

Reproduction in Free-Ranging *Propithecus verreauxi*: Estrus and the Relationship Between Multiple Partner Matings and Fertilization

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ABSTRACT Female sifaka mate selectively with one or more resident and/or non-resident males during the breeding season. Various adaptive explanations have been advanced to explain why female primates mate with multiple males including that 1) females seek to confuse paternity and thereby forestall male infanticide and/or ensure male infant care or 2) females seek to ensure fertilization. Assessing the power of fertilization insurance to explain mating patterns in females requires information on the temporal relationship between mating and ovarian hormones. The hormonal correlates of reproduction and mating in free-ranging *Propithecus verreauxi* were investigated using excreted steroids as indices of reproductive state. Solid-phase extraction and radioimmunoassay techniques were used to measure unconjugated estradiol (E_2) and progesterone (P_4) in 485 desiccated fecal samples collected from five female sifaka before and during the breeding season at Beza Mahafaly, Madagascar. Results suggest that behavioral estrus was characterized by 10- to 15-day elevations in E_2 ; hormonal activity was observed to be similar to pseudo-estrus reported for other lemur species; apparent conception was associated with sustained P_4 elevations beginning 1 to 3 days post-estrus with gestational phase elevations of E_2 beginning 42 to 45 days post-conception; and mating with multiple partners appeared not to be a prerequisite to fertilization, as conception resulted from both monoandrous and polyandrous matings. These preliminary data suggest that fertilization insurance is not adequate to explain polyandrous mating in sifaka at Beza Mahafaly.

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Female *Propithecus verreauxi* at Beza Mahafaly, Madagascar mate selectively with one or more resident and non-resident males during a 0.5- to 96-hr behavioral estrous period (Brockman, 1994). Various adaptive scenarios have been advanced to explain why females mate with multiple males including that they seek to 1) confuse paternity and thereby forestall male infanticide (Sugiyama, 1965; Goodall, 1977; Hrdy, 1979; Ru-

dran, 1979; Pereira and Weiss, 1991) or ensure male infant care (Taub, 1980; Goldizen, 1987) or 2) ensure fertilization (Milton, 1985; Sauther, 1991). In a behavioral study of mating in free-ranging *Lemur catta* it has been

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suggested (Sauther, 1991) that high-ranking females enhance the likelihood of fertilization by mating with multiple preferred males. Females exhibit estrous asynchrony within seasonal synchrony of estrus, the latter functioning to enhance female mate choice through the reduction of female-female competition for preferred mates (Pereira, 1991; Sauther, 1991). However, without information on the temporal relationship between mating and ovarian steroids, the timing of hormonal estrus among females and its relationship to mate choice, and the social factors affecting mating, the effects of multiple matings on conception or the contributions of fertility to female reproductive success cannot be measured. In this study, data on the hormonal correlates of estrus, estrous asynchrony, and on the temporal relationship between mating and ovarian steroids were obtained from free-ranging *Propithecus verreauxi* to assess the power of fertilization insurance to explain polyandrous mating patterns in female sifaka.

Verreaux's sifaka are diurnal strepsirrhine primates inhabiting riverine and dry forests of south and southwest Madagascar (Pollock, 1979; Tattersall, 1982). *Propithecus verreauxi* at Beza Mahafaly live in social groups ranging in size from two to 13 individuals, in which female philopatry and social dominance are the norm (Richard, 1987; Richard and Nicoll, 1987; Richard et al., 1991). Sifaka at this site have a 3-month breeding season beginning in January, during which most females experience single behavioral estrus periods (Brockman, 1994). Pseudo-estrus has been reported to occur in *P. verreauxi* and other strepsirrhines, behaviorally and morphologically indicated by increased attractiveness (but not receptivity) and vulval pinkening and swelling (*Propithecus verreauxi*: Richard, 1974; *Lemur catta*: Jolly, 1966; Vick, 1977; *Eulemur mongoz*: Perry et al., 1992; *Varecia variegata*: Shideler and Lindburg, 1983), and hormonally indicated by increased E_2 levels 1 month prior to behavioral estrus (Perry et al., 1992).

Fecal steroid profiles of captive *P. verreauxi* (Brockman et al., 1995) suggest that ovarian cyclicity and conception resembled other species of Lemnidae (Bogart et al.,

1977; Van Horn and Resko, 1977; Perry et al., 1992) with 5-day follicular phase increases in E_2 followed by 25- to 27-day luteal phase elevations in P_4 . Conception was associated with a 5-day 15-fold elevation in E_2 followed by a more gradual rise in P_4 (Brockman et al., 1995). Gestation was characterized by a rise in E_2 and sustained P_4 elevations occurring 43 days after the E_2 peak and concurred with gestational profiles of *Eulemur mongoz* at 30 (E_2) to 45 (P_4) days of gestation (Perry et al., 1992).

The hormonal correlates of reproduction and mating in sifaka were investigated using fecal steroids as indices of reproductive state. Fecal steroids accurately reflect ovarian function in various primates including *Propithecus verreauxi* (Brockman et al., 1995), *Callitrix jacchus* (Heistermann et al., 1992), *Pithecia pithecia* (Shideler et al., 1994), *Brachyteles arachnoides* (Strier and Ziegler, 1994), and *Papio cynocephalus* (Wasser et al., 1991; Stavisky, 1994).

The goals of this study were to 1) determine the hormonal characteristics of behavioral estrus and pseudo-estrus, 2) document within-group estrous asynchrony, 3) determine the temporal relationship between ovarian steroids and mating, and 4) elucidate the relationship between polyandrous mating and fertilization.

MATERIALS AND METHODS

Subjects and data collection

Data were collected from two habituated social groups inhabiting the Beza Mahafaly Special Reserve, Madagascar prior to and during the 1990-91 and 1992-93 breeding seasons. These data were collected from December 10, 1990 through March 14, 1991 for the Vaovao females and from December 5, 1991 through March 27, 1992 for the Vavy Masiaka females. This collection period was determined based on pre-existing birth data in the study population (Richard, personal communication). Over 644 focal animal hours (Altmann, 1974) and 485 daily fecal samples were collected on five marked females in the Vaovao and Vavy Masiaka social groups, composed, respectively, of two adult males and three adult females, and of three

adult males, one juvenile male, and two adult females.

Behavior

Data on sexual behavior were collected 7 days per week from dawn until just after sunset, using 15-min focal animal (Altmann, 1974), continuous (i.e., all occurrences recording), and ad libitum sampling techniques. Females were in view 92.28% (Vao-vao) and 89.36% (Vavy Masiaka) of the time. Fifteen-minute focal animal samples were evenly distributed among three 2-hr morning time blocks (to accommodate fecal collections) and four 1-hr afternoon/evening time blocks. Behavioral data were analyzed to focus on differences in frequencies of behavior over time. Data were checked for normality and equal variance, and depending on the results of these tests, parametric or non-parametric tests were used to examine differences and trends in the data. Behaviors recorded were mating, mounts, and attempts to mount. Mating referred to copulatory behavior in which intromission and thrusting were unambiguously observed. Mounts and mount attempts occurred for less than 3 sec without intromission and thrusting, and were typically associated with female resistance. Estrus was behaviorally defined by female willingness to mate. Pseudo-estrus was hormonally defined by elevated E_2 levels occurring one cycle length prior to behavioral estrus. Synchrony referred to the complete or partial temporal overlap of hormonal estrus of individual females, while asynchrony was the temporal non-overlap of estrus. In the absence of visible ejaculate, ejaculation was inferred based on a rapid increase in thrusts (e.g. "quiver thrusts") and then a pause just prior to the dismount, followed by intense genital grooming.

Sample collection and preservation

Morning fecal samples (1–15 g) were collected in their entirety from each female immediately after voiding, then packaged in foil, labeled, flattened to increase surface area, and dried in a Coleman oven (55°C [solar heat] to 83°C [propane heat] for 2–3 hr) within 4 hr of defecation. After drying,

the fecal samples were wrapped in plastic wrap and individually packaged with indicator silica gel packets in 4-ml zip-lock bags, labeled, and bagged again in 2.7-ml zip-lock bags with silica gel. At the end of the field season the samples were shipped to Whitten's laboratory at Emory University for extraction and radioimmunoassay analysis. The effectiveness of fecal desiccation was evaluated in a laboratory study assessing steroid degradation over time in desiccated sifaka feces. Fecal E_2 and P_4 were measured in fresh frozen samples using radioimmunoassay techniques. The samples were subsequently dried, and additional measurements were taken immediately after desiccation; these measurements were repeated at 1-, 2-, and 3-week intervals.

Hormone assay

Each females' dried fecal pellets from a single void were pooled and pulverized. Unconjugated fecal E_2 and P_4 were quantified using solid phase extraction and radioimmunoassay (RIA) procedures previously validated for this species (Brockman et al., 1995).

Fecal steroid extraction. A 0.1-g sample of dried feces was homogenized in 2 ml methanol:acetone (8:2, v/v) for 30 sec at 1,300 rpm. The homogenate was filtered by layering onto a 0.2- μ m G Prep PTFE filter (Gelman Sciences, Ann Arbor, MI) and centrifuged at 1,500g for 5 min. The filtrate was solid phase extracted on small columns of reversed-phase octadecylsilane (C18) bonded to silica (Baxter/Burdick & Jackson, Muskegon, MI) using the methods of Shackleton and Whitney (1980). The column was primed with 2 ml of methanol, followed by 5 ml of deionized water. The sample was then layered onto the column, and the column was washed with 5 ml of water. The steroid extract was then diluted with 3 ml methanol. Recovery data indicated that an average of $68 \pm 5\%$ of E_2 and P_4 were eluted in the first 2 ml of methanol.

Estradiol radioimmunoassay. The E_2 RIA procedures followed the microassay procedures developed by Worthman et al. (1990). This protocol was a modification of the Pan-

TABLE 1. Progesterone and estradiol recoveries over time in desiccated feces from *Propithecus verreauxi*

| | Fresh frozen (ng/g) | Post-dry (%) (ng/g) | 3 weeks (%) (ng/g) |
|-----------------------|------------------------|------------------------|-----------------------|
| Progesterone | | | |
| Low ¹ | 8.0 | 4.4 (55) ⁴ | — ⁵ |
| | 8.0 | 5.6 (70) | — |
| | 12.0 | 5.4 (45) | 3.3(28) |
| | 17.0 | 5.6 (33) | 5.0 (29) |
| Recovery ² | 43.5 ± 10.6 | 22.0 ± 0.0 (50.5) | 10.0 ± 2.8 (23) |
| High ³ | 47.0 | 12.6 (27) | 11.0 (23) |
| | 20.5 | 14.4 (80) | 9.0 (44) |
| Recovery | 100.5 ± 57.3 | 43.5 ± 78 (43.3) | 18.0 ± 1.4 (17.9) |
| Estradiol | | | |
| Low | 122.0 | 81.6 (67) | 142.0 (116) |
| | 93.0 | 74.4 (80) | 97.3 (105) |
| | 64.0 | 48.8 (76) | — |
| | 54.0 | 54.8 (100) | — |
| Recovery | 0.36 ± 0.06 | 0.32 ± 0.02 (86.4) | 0.28 ± 0.08 (75.7) |
| High | 937.0 | 659.6 (70) | 886.7 (95) |
| | 801.0 | 631.2 (79) | 1,300.0 (162) |
| Recovery | 2.60 ± 0.30 | 2.00 ± 0.10 (75.4) | 2.00 ± 0.50 (75.5) |

¹ Samples with lowest steroid levels.² Mean recoveries ± standard deviation.³ Samples with the highest steroid levels.⁴ Percentage of steroid recovered.⁵ Samples discarded due to defective extraction cartridge.

tex Direct ¹²⁵I Estradiol 174M kit (Santa Monica, CA). Working buffer was 0.1% gelatin phosphate-buffered saline (PBS; pH 7.4). An aliquot of fecal extract was evaporated under nitrogen and reconstituted in PBS at a 1:10 dilution. ¹²⁵I Estradiol tracer (50 µl) and 300 µl of antiserum diluted 1:4 in working buffer were added to 200-µl aliquots of the standards (diluted 1:5 to give concentrations of 1–1024 pg/ml), samples, and controls (diluted 1:5). Each was vortexed and incubated overnight at room temperature. The following morning, 500 µl PEG second antibody, diluted 1:2, was added, and the incubates were vortexed, incubated at additional 20 min at room temperature, and centrifuged at 1,500g for 60 min at 4°C. The supernatant was decanted, and the radioactivity of the precipitate was determined by 10-min counts in a Packard RIASTAR gamma counter (Packard, Downer's Grove, IL) with RIASMART and Expert QC software.

Progesterone radioimmunoassay. The P₄ RIA was performed as previously described

in Brockman et al. (1995). These procedures followed the microassay procedures developed by Worthman and Stallings (unpublished), using reagents from the Pantex (Santa Monica, CA) ¹²⁵I P₄ Kit for serum determinations. Working buffer was (pH 7.4) with 0.1% bovine serum albumin and 0.1% sodium azide as preservative (PBS-BSA). An aliquot of fecal extract was evaporated under nitrogen and reconstituted 1:1 in PBS-BSA. ¹²⁵I P₄ tracer (100 µl), and 100 µl of antiserum diluted 1:6 with PBS-BSA were added to aliquots (100 µl) of the standards (diluted 1:5 to give concentrations of 0.04–16 ng/ml), samples, and controls (Bio-Rad, ECS Division, Anaheim, CA, diluted 1:5). Each was vortexed and incubated overnight at room temperature. The following morning, second antibody (Pantex, 100 µl) was added, and the incubates were vortexed, incubated an additional hour at room temperature, and then centrifuged at 1,500g for 1 hr at 22°C. Following decanting of the supernatants, radioactivity of the precipitate was determined by 10-min counts in the gamma counter.

TABLE 2. Mean estradiol and progesterone concentrations of five female *Propithecus verreauxi* for 12-day intervals during the estrous cycle, where estrus = interval -6 to +6

| Sample interval | Estradiol (ng/g) | | | Progesterone (ng/g) | | |
|-----------------------|------------------|----------------|----------------|---------------------|----------------|----------------|
| | Mean (SEM) | N ¹ | N ² | Mean (SEM) | N ¹ | N ² |
| -79 to -90 | 6.46 ± 1.21 | 1 | 2 | 101.7 ± 80.2 | 1 | 2 |
| -67 to -78 | 4.53 ± 0.36* | 1 | 12 | 212.1 ± 13.0 | 1 | 12 |
| -55 to -66 | 6.83 ± 1.90* | 1 | 12 | 284.3 ± 16.6 | 1 | 12 |
| -43 to -54 | 8.38 ± 2.82* | 1 | 11 | 240.8 ± 14.1 | 1 | 11 |
| -31 to -42 | 8.33 ± 1.35* | 3 | 23 | 313.0 ± 27.6 | 3 | 23 |
| -19 to -30 | 11.43 ± 1.99* | 5 | 50 | 315.8 ± 20.4 | 5 | 50 |
| -7 to -18 | 15.21 ± 1.71** | 5 | 57 | 358.6 ± 29.5 | 5 | 57 |
| -6 to +6 ³ | 26.71 ± 4.68 | 5 | 64 | 383.6 ± 34.6 | 5 | 64 |
| +7 to +18 | 9.77 ± 0.84* | 5 | 60 | 637.8 ± 58.0* | 5 | 60 |
| +19 to +30 | 20.09 ± 3.64 | 5 | 59 | 578.6 ± 52.9** | 5 | 59 |
| +31 to +42 | 21.34 ± 3.22 | 5 | 47 | 778.2 ± 80.0* | 5 | 47 |
| +43 to +54 | 288.54 ± 48.87* | 4 | 35 | 1,480.5 ± 220.7* | 4 | 35 |
| +55 to +66 | 196.25 ± 65.06* | 2 | 11 | 611.2 ± 236.9 | 2 | 11 |

¹ No. of females sampled per interval.² Total No. of fecal samples per interval.³ Estrous interval.* $P < .001$ vs. estrus (-6 to +6).** $P < .01$ vs. estrus (-6 to +6).

RESULTS

Fecal sample preservation

Radioimmunoassay results assessing steroid degradation over time in aliquots from the same void of desiccated feces showed that in contrast to estradiol (E_2), which was fairly stable, progesterone (P_4) values did appear to decline with time although the time effects were not significant (one-way repeated measures ANOVA: E_2 : $F = 4.04$, $n = 16$, $P = .06$; P_4 : $F = 3.70$, $n = 16$, $P = .07$, Table 1).

Hormonal characteristics of estrus and pseudo-estrus

Estrus. In most cases, behavioral signs of willingness to mate were associated with marked 10- to 15-day elevations in E_2 levels. Estrous elevations in E_2 lasted 10 to 15 days (mean = 12 days), representing 2- to 14-fold increases above baseline E_2 levels which averaged 6.3 ng/g. Consequently, 12-day intervals were used to assess fecal steroids in and across females. For all five females combined, mean E_2 and P_4 values were significantly different between estrous and post-estrous 12-day intervals (Mann-Whitney rank-sum test: E_2 : $T = 2825.5$, $P < .001$; P_4 : $T = 4407.0$, $P = .001$), and E_2 , but not P_4 , was significantly different between pre-estrous and estrous 12-day intervals (Mann-Whitney rank-sum test: E_2 : $T = 2991.5$, $P = .012$; Table 2; Fig. 1). Elevations were

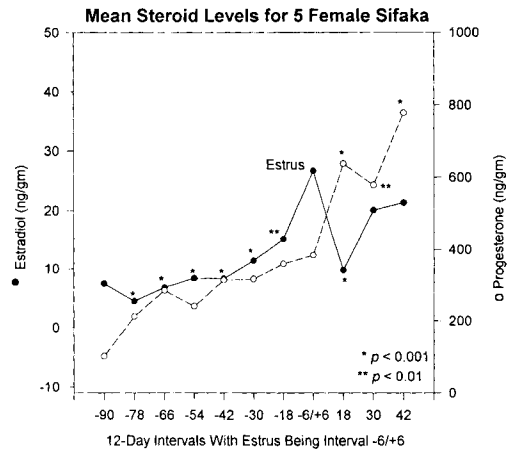


Fig. 1. Combined 12-day mean estradiol and progesterone levels for five female *Propithecus verreauxi* (*denotes significant differences between estrous [interval -6/+6] and pre-estrous and post-estrous steroid levels).

defined in terms of 12-day running averages wherein mean E_2 values consistently increased beginning 30 days prior to estrus. In all but one case, females' estrus E_2 levels were elevated two- to 12-fold above the 19- to 30-day values (Table 2). Female 107, however, exhibited highest E_2 values 1 month prior to estrus associated with brief mating attempts by resident males (see below).

TABLE 3. Estradiol peaks, elevation durations, and sexual behavior in Vavy Masiaka females¹

| Date (December 1991 to March 1992) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|------------------------------------|-------------------|----|----|----|----|-------------------|----|----|----|----|----|-----|---|---|----|---|---|---|---|-------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|------|----|----|----|----|
| Female | 12/25 | 26 | 27 | 28 | 29 | 1/22 | 23 | 24 | 29 | 30 | 31 | 2/1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 3/19 | 20 | 21 | 22 | 26 |
| F107 | <--^----->(4) | | | | | <-----^----->(14) | | | | | | | | | | | | | | #-----^----->(10) | | | | | | | | | | | | | | | | | | | |
| | Mt | | | | | Mt | | | | | | | | | | | | | | MT | | | | | | | | | | | | | | | | | | | |
| F36 | #-----^----->(15) | | | | | | | | | | | | | | MT | | | | | | | | | | | | | | | | | | | | | | | | |

¹ < >, Non-estrus-related estradiol (E₂) elevations; (), E₂ elevation duration in days; #, Estrus-related E₂ elevations; ^, E₂ peak; MT, mating; copulation with intromission and thrusting; Mt, mounts: 3-sec mounts with no intromission, associated with female resistance to males.

TABLE 4. Estradiol peaks, elevation durations, and sexual behavior in Vaovao females¹

| Date (January 1991) | | | | | | | | | | | | | | | | | | | | | | | |
|---------------------|-------------------|---|---|---|---|---|----|----|----|----|-------------------|----|----|----|----|----|----|----|----|--|--|--|--|
| Female | 1 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | | | | |
| <i>F20</i> | #-----^-----#(10) | | | | | | | | | | MT MT | | | | | | | | | | | | |
| <i>F19</i> | #-----^-----#(11) | | | | | | | | | | MT MT MT | | | | | | | | | | | | |
| <i>F80</i> | #-----^-----#(13) | | | | | | | | | | #-----^-----#(Mt) | | | | | | | | | | | | |
| | Mt Att. . . . Mt | | | | | | | | | | | | | | | | | | | | | | |

¹ #, Estrus-related estradiol (E₂) elevations; (), E₂ elevation in days; ^, E₂ peak; MT, mating: copulation with intromission and thrusting; Mt and Mt Att, mounts and mount attempts: no intromission, associated with female willingness to mate and intense copulatory harassment.

Pseudo-estrus. Two females exhibited 1- and 4-day elevations in E₂ 27 and 31 days prior to behavioral estrus, similar to pseudo-estrous E₂ elevations seen in captive *Eulemur mongoz* (Perry et al., 1992).

Estrous asynchrony

Hormonal data from this study showed that estrus was asynchronous in Vavy Masiaka and synchronous in Vaovao. The Vavy Masiaka females exhibited no temporal overlap in E₂ peaks or in sexual activity (i.e., mounts and mating, Table 3). While there was some overlap in E₂ elevations, estrous elevations in E₂ were non-overlapping, indicating that estrus was asynchronous in this social group. In contrast, the Vaovao females exhibited temporal overlap in E₂ elevations and in the case of females 19 and 80, E₂ peaks occurred on the same day. Although mounts and copulations were non-overlapping, male attempts to mount F80 coincided with F19's mating activity (Table 4). Estrus-related intrasexual competition occurred in both the synchronous and asynchronous social groups, associated with male guarding and copulatory harassment by males and females.

The temporal relationship between ovarian steroids and mating

Estradiol and P₄ were quantified spanning a 146-day period from 80 days prior to estrus (i.e., F107) to day 66 post-estrus (i.e., F20). Figures 2–6 show E₂ and P₄ profiles for the five females in this study. In all but one case, sifaka females mated when E₂ levels were elevated substantially above baseline levels. Although F80 was never seen mating, receptivity to mounts and mount attempts were associated with elevated E₂ levels, suggesting that an undetected copulation may have occurred at this time. Previous studies of captive primates show that while fecal levels of ovarian steroids accurately reflect serum steroid levels, a 1- to 3-day lag occurs between serum and fecal concentrations of ovarian steroids (Wasser et al., 1988; Shideler et al., 1993; Stavisky et al., 1994). In this study, most matings occurred during the 10- to 15-day elevations in E₂, the latter theoretically corresponding to both serum and fecal concentrations of periovulatory E₂. In the profiles which follow, conception and gestation were inferred based on similarities to fecal profiles reported for captive *P. ver-*

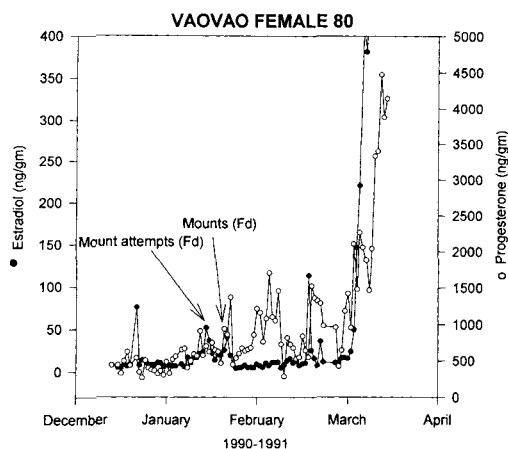


Fig. 2. Estradiol and progesterone profiles for Vao-vao female 80 and associated sexual behavior with male Fd.

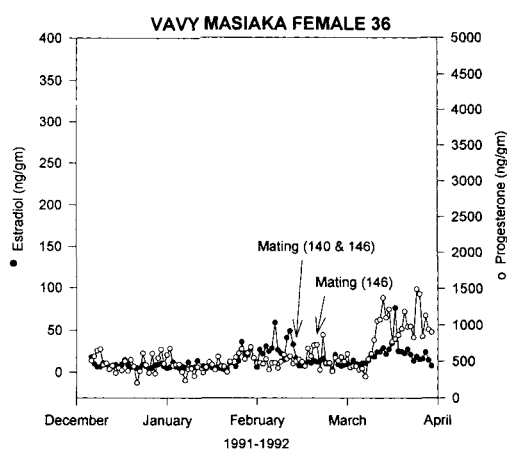


Fig. 5. Estradiol and progesterone profiles for Vavy Masiaka female 36 and associated sexual behavior with males 146 and 140.

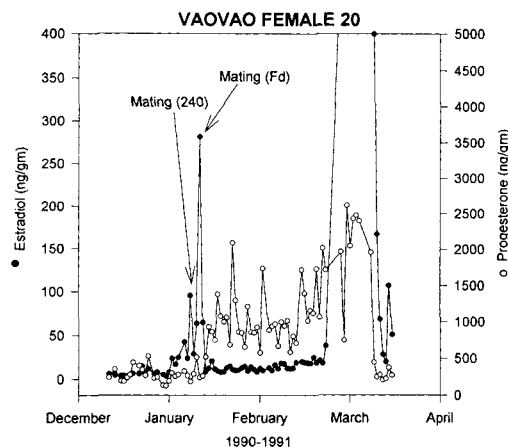


Fig. 3. Estradiol and progesterone profiles for Vao-vao female 20 and associated sexual behavior with males 240 and Fd.

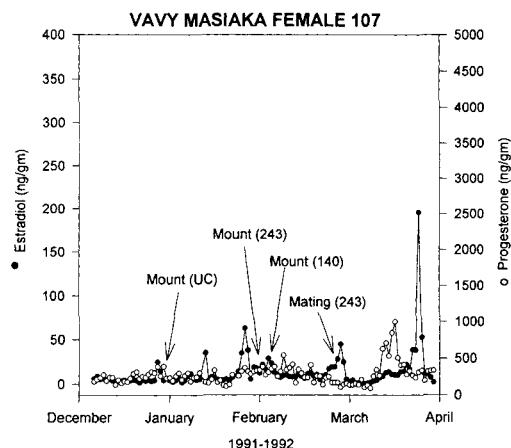


Fig. 6. Estradiol and progesterone profiles for Vavy Masiaka female 107 and associated sexual behavior with males UC, 140, and 243.

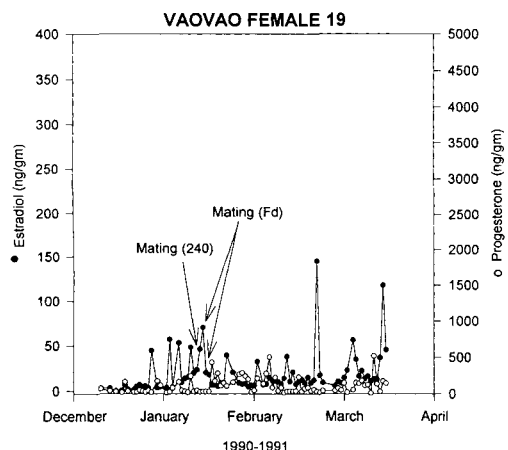


Fig. 4. Estradiol and progesterone profiles for Vao-vao female 19 and associated sexual behavior with males 240 and Fd.

reauxi who gave birth (see opening paragraphs; Brockman et al., 1995) and from other conceptive lemurs (Perry et al., 1992).

In F80's profile (Fig. 2) mounts and mount attempts by male Fd (MFd) were associated with the mid-January 13-day twofold elevation in E_2 . A large 50-fold E_2 elevation above baseline occurred in early March, beginning 43 days after the January 19 mounts. Two additional single-day elevations also occurred 31 and 28 days prior to and after estrus on December 20 and February 14, the former suggestive of a possible pseudo-estrus. Progesterone elevations began 3 days

after the January 19 E_2 elevation and lasted 46 days when sampling terminated. The late-February elevations in ovarian steroids were substantially higher than captive pregnancy levels (Brockman et al., 1995), suggesting that F80 may have conceived during her first estrous period.

Female 20 mated with both resident males during the 10-day 12-fold E_2 elevation in early January (Fig. 3). A second 133-fold elevation occurred 42 days after estrus and lasted 14 days before dropping precipitously to pre-estrous levels. Progesterone elevations began immediately after the sharp January 9 rise and lasted 54 days before dropping to pre-estrous levels. The late-February P_4 elevation was similar to that reported for captive pregnant sifaka (Brockman et al., 1995), suggesting that F20 may also have conceived during her first estrous period. The precipitous drop in steroid levels in early March, however, suggests that an abortion may have occurred 58 to 61 days post-conception.

Female 19 copulated with both resident males during her 11-day sevenfold E_2 elevation in mid-January (Fig. 4). Several additional, but more abbreviated, pre- and post-estrous elevations occurred lasting 1 to 2 days unaccompanied by observations of sexual behavior. Elevations in P_4 began immediately after the sharp January E_2 rise and lasted 23 days, before resuming pre-estrous levels. The mid-March elevations in ovarian steroids 46 days later may be indicative of the onset of cyclicity.

Female 36 mated with two of the three resident males during her variable 15-day fourfold E_2 elevation in mid-February (Fig. 5). She copulated again 8 days later when E_2 levels were just above baseline levels. A second 7-day, 13-fold rise in E_2 occurred 36 days later on March 15. Progesterone elevations began 2 days after the February 9 E_2 rise and lasted 43 days until sampling terminated. The mid-March elevation in ovarian steroids was one-third that reported for captive pregnant sifaka (Brockman et al., 1995), although it is not clear that the E_2 elevation was sustained. However, the late-February to late-March portion of the profile resembles F80's pregnancy profile 28 days post-

estrus and suggests that F36 may have conceived in mid-February.

Female 107 mated with one of the three resident males during the 10-day E_2 elevation on February 23 (Fig. 6). Three additional 4- to 14-day E_2 elevations occurred in late-December, January, and March, representing two- to 14-fold elevations above baseline levels. Brief mounts by two other resident males were associated with the December and January E_2 elevations, indicating that cyclic follicular activity may have been occurring prior to estrus. Except for the mid-March 19-day elevation, F107's P_4 profile remained fairly constant throughout most of the sampling period, with baseline levels equal to those reported for non-breeding females (Brockman et al., 1995).

Polyandrous mating and fertilization

Two of the three females who appeared to exhibit hormonal evidence of conception (F20, F36) mated with two resident males, while the third female (F80) was mounted by the younger resident male, having steadfastly refused to mate with the group's older resident male (Table 5). Female 20 mated 14 times with males 240 and Fd, five copulations being ejaculatory, while F36 mated three times with males 146 and 140, with two copulations resulting in ejaculation. Female 80 was mounted four times by male Fd, associated with intense guarding and harassment by M240. None of the females was observed with an infant the following birth season.

DISCUSSION

These results suggest that reproductive state (i.e., estrus and conception) of free-ranging female sifaka can be reliably assessed using radioimmunoassay (RIA) techniques in conjunction with observations of sexual behavior.

Three sifaka females (F80, F20, F36) experienced gradual two- to 12-fold estrous increases in E_2 over 10- to 15-day periods, followed 1 to 3 days later by sustained elevations in P_4 . Although estrous elevations in E_2 were two to three times longer than those observed in captive sifaka who subsequently conceived (Brockman et al., 1995), the patterning of ovarian steroid secretion was sim-

TABLE 5. Mating in Vaovao and Vavy Masiaka social groups for which there is hormonal evidence of outcome

| Females | Males | Date | Number of copulations | Number of ejaculations | Outcome |
|-----------------------|-------|---------|-----------------------|------------------------|-------------------------|
| Vaovao: 1990-91 | | | | | |
| 20 | 240 | Jan. 7 | 11 | 3 | Conception ¹ |
| | Fd | Jan. 9 | 3 | 2 | |
| 19 | 240 | Jan. 11 | 7 | 1 | No evidence |
| | Fd | Jan. 12 | 2 | 0 | of conception |
| | | Jan. 13 | 3 | 1 | |
| 80 | Fd | Jan. 19 | 4 (mounts) | | Conception |
| Vavy Masiaka: 1991-92 | | | | | |
| 36 | 140 | Feb. 10 | 1 | 1 | Conception |
| | 146 | Feb. 10 | 1 | 1 | |
| | 146 | Feb. 18 | 1 | 0 | |
| 107 | 243 | Feb. 23 | 3 | 1 | No evidence |
| | | | | | of conception |

¹ Refers to conception with male(s) identified in column 1.

ilar, indicating that two of these profiles may represent later gestational stages of conception. The reasons why estrus may be longer in free-ranging than in captive sifaka females are unclear, but studies of captive *Microcebus murinus* suggest that social group size influences the duration of estrous cycles so that cycle lengths are increased in group-housed females (Perret, 1982). Although not indicative of gestation, F36's P₄ profile resembled F80's early conceptive profile before gestational increases in E₂ and P₄ might be expected to occur.

All of these females appeared to conceive during their first estrous period although none was observed with infants the following birth season. Explanations for this "absence of infants" in these three conceptive females include abortion, missed data, and perinatal mortality. Data on abortion in strepsirhines are few. Except for the inferred abortion reported on here (Results: F20 and below), data on abortion in lemurs are limited to a study of *Microcebus murinus* showing an 18.1% to 35.2% spontaneous abortion rate in captive females (Perret, 1982). Abortion in captive sifaka is reportedly rare and may be associated with translocation stress (Haring, personal communication).

Hormonal data suggest that while abortion may explain why F20 was not observed with an infant, it is not adequate to explain the lack of infants for the two other apparently conceptive females. Alternatively, we propose that missed data and perinatal mortality are the best explanations for the ab-

sence of births reported for females 80 and 36. Sifaka at Beza Mahafaly have been censused annually since 1984, data being typically gathered toward the end of the birth season in August (Richard et al., 1991). Extrapolations from observations of mating and a 163-day gestation period (Petter-Rousseaux, 1962) indicate that female 80 and 36 would have delivered during the 4th week of June 1991 and the 3rd week of July 1992, respectively, 2-5 weeks before the onset of the census period. This observation gap combined with demographic data showing an average 61% (1984-1988 range: 30-80%) 1st-year infant survival rate (Richard et al., 1991) suggests that if these females did give birth to term infants unobserved, the infants could also have died unnoticed soon thereafter. The difficulty of establishing conception from demographic data which are not typical of wild primate populations provides a strong argument for further applications of fecal steroid techniques.

Female 19's and 107's steroid profiles were distinctively different. Female 19's profile was erratic, showing no clear pattern of E₂ or P₄ secretion. Similar variation in E₂ secretion has been observed in a captive female who mated but failed to conceive (Brockman et al., 1995). While F107's 27- to 31-day estrus-related cycles in E₂ secretion indicated adequate follicular function, they were unaccompanied by P₄ cyclicity, suggesting that this female may have been anovulatory or just beginning ovarian cyclicity when sampling terminated.

Hormonal data show that while only 60% of the females in this study apparently conceived, the small sample size dictates caution in extending these results to extrapolations about conception rates in this population. Available data on conception in strepsirrhine primates derive from cytological and morphological evidence of cycling and births in captive *Eulemur mongoz* (Perry et al., 1992) and *Varecia variegata* (Brockman et al., 1987). These studies show that conception rates range from 58% to 64% in mongoose lemurs ($n = 24$; Perry et al., 1992) and ruffed lemurs ($n = 26$; Brockman et al., 1987), respectively. Although preliminary, the hormonal results from this study concurred with the trends reported above in captive lemurs, tentatively suggesting that females at Beza Mahafaly may experience variations in their ability to conceive. This hypothesis is supported by recent demographic data suggesting that females may exhibit variance in lifetime reproductive success associated with decreased fertility rates among older age-class females, a decline in successful reproduction (i.e., the proportion of females giving birth to infants that survived the 1st year) with increasing age, and variable cumulative reproductive success (i.e., the average number of surviving offspring born per birth season) within age classes (Richard et al., 1991). Ongoing and anticipated studies of this population's demographic and reproductive patterns will clarify these preliminary results.

Studies of captive *Eulemur mongoz* show that pseudo-estrus is hormonally characterized by E_2 elevations unaccompanied by behavioral estrus occurring one cycle length prior to ovulatory cycles (Perry et al., 1992). In this study, only two females (F80, F107) exhibited hormonal profiles clearly consistent with the hypothesis of pseudo-estrus. Females 80 and 107 exhibited 1- to 4-day elevations in E_2 27 to 31 days prior to behavioral estrus unaccompanied by elevations in P_4 , the latter indicative of an anovulatory cycle.

Most females in this study resembled captive lemurs (Perry et al., 1992; Shideler et al., 1983; Brockman et al., 1995) in that behavioral estrus was associated with elevated E_2 levels. However, the Vavy Masiaka fe-

males also exhibited sexual behavior in the absence of elevated ovarian hormones, coincident with the immigration of a new resident male. Eighty percent of F107's sexual presents were non-estrus-related, being directed toward M243 in the time period following his transfer into the group. Similarly, one-third of F36's matings occurred in the absence of E_2 elevations, associated with copulations with the group's recently peripheralized M146 out of sight of the new central male. These observations suggest that hormone-sexual behavior disjunctions may occur in sifaka associated with changes in group male membership and female preferences for certain males.

In this study, timing of estrus varied between social groups. Estrus was asynchronous within each social group based on observations of successful mating, whereas estrous elevations in E_2 were synchronous in Vaovao females (Table 4) and asynchronous in Vavy Masiaka females (Table 3). Previous behavioral studies of captive (Pereira, 1991) and free-ranging *Lemur catta* (Sauther, 1991) suggest that within-group estrous asynchrony reduces female-female competition and enhances the ability of females to mate with multiple preferred males. Pereira (1991) argues that estrous asynchrony, defined by vulval morphology and mating, is deterministic, social entrainments and pheromone-based signal-response systems functioning to mediate estrous cycles in ringtailed lemur social groups. The results of this study suggest, however, that the criteria used to measure estrous synchrony/asynchrony are crucial to its determination, and that hormonal data may more accurately reflect periovulatory events than vulval morphology or observations of mating (Pereira, 1991).

Within-group estrous synchrony/asynchrony per se appeared not to affect multiple mating by females. Two of the three hormonally synchronous Vaovao females mated polyandrously (F20, F19), while the hormonally asynchronous Vavy Masiaka females mated monoandrously (F107) and polyandrously (F36). Rather, mating with preferred mates was limited by intrasexual competition associated with male guarding and copulatory harassment by males and females

and by female aversions to mating with certain males.

The behavior and hormonal data from this small sample of females do not support fertilization insurance as an explanation for polyandry in sifaka. Two of three females (F20 and F36) who copulated two or more times with more than one resident male apparently conceived, while the other female (F80) may have conceived after having been mounted by a single male. In the latter case, the hormonal data suggest that F80 probably conceived during F19's estrous period when male Fd was observed to mount F80 several times over her 13-day hormonal estrous period. An alternative explanation for polyandry in sifaka is that females are seeking to confuse paternity to forestall male infanticide. Support for this idea comes from recent observations of male infanticide in *P. diadema* at Ranomafana (Wright, 1995) and *P. verreauxi* at Beza Mahafaly (Kubzdela, personal communication). Further support for the infanticide hypothesis is provided by the behavior-hormone disjunctions observed in this study, suggesting that females exhibit non-estrus-related sexual behavior toward newly immigrated and peripheral males to establish and maintain social bonds and guard against the possibility of male infanticide.

Finally, this study suggests that negative reproductive consequences may accrue to pregnant females who engage in agonistic encounters during the breeding season. Female 20's steroid profile appeared to show that her pregnancy terminated in early March, the latter coinciding with observations of marked increases in inter-group agonism. Although circumstantial, these data offer additional avenues of inquiry for identifying specific social constraints affecting reproductive fitness in free-ranging female sifaka.

In conclusion, 1) behavioral estrus was characterized by 10- to 15-day elevations in E_2 ; 2) hormonal activity was observed similar to pseudo-estrus reported for other lemur species; 3) apparent conception was associated with sustained P_4 elevations beginning 1 to 3 days post-estrus with gestational phase elevations of E_2 beginning 42 to 45 days post-conception; and 4) mating with

multiple partners did not appear to be a prerequisite for fertilization, as conception resulted from both monoandrous and polyandrous matings.

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LITERATURE CITED

- Altmann J (1974) Observational study of behavior: sampling methods. *Behaviour* 49:227-267.
- Bogart MH, Kumamoto A, and Benirschke K (1983) A comparison of the reproductive cycles of three species of *Lemur*. *Folia Primatol.* (Basel) 28:134-143.
- Brockman DK (1994) Reproduction and mating system of Verreaux's sifaka, *Propithecus verreauxi*, at Beza

- Mahafaly, Madagascar. Ph.D. dissertation, Yale University, New Haven, CT.
- Brockman DK, Willis MS, and Karesh WB (1987) Management of husbandry of ruffed lemurs, *Varecia variegata*, at the San Diego Zoo. II. Reproduction, pregnancy, parturition, litter size, infant care, and reintroduction of hand-raised infants. *Zoo Biol.* 6:349–363.
- Brockman DK, Whitten PL, Russell E, Richard AR, and Izard MK (1995) Application of fecal steroid techniques to the reproductive endocrinology of female Verreaux's sifaka, *Propithecus verreauxi*. *Am. J. Primatol.* 36:313–325.
- Goldizen AW (1987) Facultative polyandry and the role of infant-carrying in wild saddle-backed tamarins (*Saguinus fuscicollis*). *Behav. Ecol. Sociobiol.* 20:99–109.
- Goodall J (1977) Infant killing and cannibalism in free-living chimpanzees. *Folia Primatol.* (Basel) 28:259–282.
- Heistermann H, Tari S, and Hodges JK (1992) Monitoring ovarian function in Callitrichidae by faecal steroid analysis. *Bio. Reprod.* 46:30.
- Hrdy SB (1979) Infanticide among animals: a review, classification, and examination of the implications for the reproductive strategies of females. *Ethol. Sociobiol.* 1:13–40.
- Jolly A (1966) *Lemur Behavior: A Madagascar Field Study*. Chicago: University of Chicago Press.
- Milton K (1985) Mating patterns of woolly spider monkeys, *Brachyteles arachnoides*: Implications for female choice. *Behav. Ecol. Sociobiol.* 17:53–59.
- Pereira ME (1991) Asynchrony within estrous synchrony among ringtailed lemurs (Primates: Lemnidae). *Psychol. Behav.* 49:47–52.
- Pereira ME, and Weiss ML (1991) Female mate choice, male migration, and the threat of infanticide in ringtailed lemurs. *Behav. Ecol. Sociobiol.* 29:141–152.
- Perry JM, Izard MK, and Fail PA (1992) Observations on reproduction, hormones, copulatory behavior, and neonatal mortality in captive *Lemur mongoz* (mongoose lemur). *Zoo Biol.* 11:81–97.
- Perret M (1982) Influence du groupement social sur la reproduction de la femelle de *Microcebus murinus* (Miller 1777). *Z. Tierpsychol.* 60:47–65.
- Petter-Rousseaux A (1962) Recherches sur la biologie de la reproduction des primates inférieurs. *Mammalia* 26:1–88.
- Pollock JI (1979) Spatial distributions and ranging behavior in lemurs. In GA Doyle and RD Martin (eds.): *The Study of Prosimian Behavior*. New York: Academic Press, pp. 359–409.
- Richard AR (1974) Patterns of mating in *Propithecus verreauxi*. In RD Martin, GA Doyle, and AC Walker (eds.): *Prosimian Biology*. London: Duckworth Press, pp. 49–75.
- Richard AF (1987) Malagasy prosimians: Female dominance. In BB Smuts, DL Cheney, RM Sayfarth, RW Wrangham, and TT Struhsaker (eds.): *Primate Societies*. Chicago: University of Chicago Press, pp. 25–33.
- Richard AF, and Nicoll ME (1987) Female dominance and basal metabolism in a Malagasy primate, *Propithecus verreauxi*. *Am. J. Primatol.* 12:309–314.
- Richard AF, Rakotomanga P, and Schwartz M (1991) Demography of *Propithecus verreauxi* at Beza Mahafaly, Madagascar: sex ratio, survival, and fertility, 1984–1988. *Am. J. Phys. Anthropol.* 84:307–322.
- Rudran R (1979) The demography and social mobility of a red howler (*Alouatta seniculus*) population in Venezuela. In JF Eisenberg (ed.): *Vertebrate Ecology in the Northern Neotropics*. Washington DC: Smithsonian Institution Press, pp. 107–126.
- Sauther ML (1991) Reproductive behavior of free-ranging *Lemur catta* at Beza Mahafaly Special Reserve, Madagascar. *Am. J. Phys. Anthropol.* 84:463–477.
- Shackleton CHL, and Whitney JO (1980) Use of Sep-pak cartridges for urinary steroid extraction: evaluation of the method for use prior to gas chromatographic analysis. *Clin. Chim. Acta* 107:231–243.
- Shideler SE, and Lindburg DG (1983) Selected aspects of *Lemur variegatus* reproductive biology. *Zoo Biol.* 1:127–134.
- Shideler SE, Lindburg DG, and Lasley BL (1983) Estrogen-behavior correlates in the reproductive physiology and behavior of the ruffed lemur (*Lemur variegatus*). *Horm. Behav.* 17:249–263.
- Shideler SE, Ortuño AM, Morán FM, Moorman EA, and Lasley BL (1993) Simple extraction and enzyme immunoassays for estrogen and progesterone metabolites in the feces of *Macaca fascicularis* during non-conceptive and conceptive ovarian cycles. *Biol. Reprod.* 48:1290–1298.
- Shideler SE, Savage A, Ortuño AM, Moorman EA, and Lasley BL (1994) Monitoring female reproductive function by measurement of fecal estrogen and progesterone metabolites in the White-faced saki (*Pithecia pithecia*). *Am. J. Primatol.* 32:95–108.
- Stavisky RC (1994) Socioendocrinology: noninvasive techniques for monitoring reproductive function in captive and free ranging primates. Ph.D. dissertation, Emory University, Atlanta, GA.
- Stavisky RC, Wilson M, Russell E, and Whitten PL (1994) Validation of the fecal extraction method: correlation of serum and fecal endocrine profiles in rhesus macaques. *Am. J. Phys. Anthropol.* 18:186.
- Strier KB, and Ziegler TE (1994) Insights into ovarian function in wild Muriqui monkeys (*Brachyteles arachnoides*). *Am. J. Primatol.* 32:31–40.
- Sugiyama Y (1965) On the social change of hanuman langurs (*Presbytis entellus*) in their natural conditions. *Primates* 6:213–247.
- Tattersall I (1982) *The Primates of Madagascar*. New York: Columbia Press.
- Taub DM (1980) Female choice and mating strategies among wild Barbary macaques (*Macaca sylvanus*). In D Lindburg (ed.): *The Macaques: Studies in Ecology, Behavior and Evolution*. New York: Van Nostrand Reinhold Press, pp. 287–345.
- Van Horn RN and Resko JA (1977) The reproductive cycle of the ring-tailed lemur (*Lemur catta*): sex steroid levels and sexual receptivity under controlled photoperiods. *Endocrinology* 101:1579–1586.
- Vick LG (1977) The role of interindividual relationships in two troops of captive *Lemur fulvus*. Ph.D. dissertation, University of North Carolina, Chapel Hill, NC.
- Wasser SK, Risler L, and Steiner RA (1988) Excreted steroids in primate feces over the menstrual cycle and pregnancy. *Biol. Reprod.* 39:862–872.

- Wasser SK, Monfort SL, and Wildt DE (1991) Rapid extraction of faecal steroids for measuring reproductive cyclicity and early pregnancy in free-ranging yellow baboons (*Papio cynocephalus*). *J. Reprod. Fertil.* 92:415–423.
- Worthman CM, Stallings JF, and Hoffman LF (1990) Sensitive salivary estradiol assay for monitoring ovarian function. *Clin. Chem.* 36:1769–1773.
- Wright PC (1995) Demography and life history of free-ranging *Propithecus diadema edwardsi* in Ranomafana National Park, Madagascar. *Int. J. Primatol.* 16:835–854.